THE CONDUCTION OF THE NERVOUS IMPULSE THROUGH THE PEDAL GANGLION OF MYTILUS.

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The object of these experiments was to determine the change in the rate and nature of nervous conduction through the pedal ganglion of *Mytilus*. The large Pacific Coast mussel, *M. californianus* Conrad, was used in the early experiments which were made at the Hopkins Marine Station of Stanford University during the summer of 1926. The latter experiments were made at the Marine Biological Laboratory during the summer of 1928, using *M. edulis* L. The mussel was used rather than *Ensis* (*cf.* Drew, 1908) because the former permitted an independent nerve muscle preparation of the foot while the cerebral ganglia must be included in a similar preparation of the latter in order to obtain a reflex response. Nerve conduction through the ganglion was much slower with both species of animals than the conduction in the pedal nerve. This difference was greatly reduced after a solution of strychnine was placed on the ganglion. Camphor also removed the inhibiting effect of the ganglion, and atropine caused a reversal of the contractions of the longitudinal and circular muscles of the foot.

I.

The foot of *M. californianus* obtained by severing it from the animal makes an excellent nerve-muscle preparation. It quickly relaxes from the stimulation of the cut and may be pinned firmly to a wax-bottomed dish by two pins placed about 8 mm. from its distal end. The pedal nerve and ganglion may be easily exposed by a cut through the dermis the length of the foot. After the position of the nerve is familiar it is not necessary to make cuts other than at the points of stimulation.

In the following experiments the nerve was not exposed further

than by carefully pushing the points of the platinum electrodes through the skin, so as not to injure the nerve, until the records were completed. After that the ganglion was exposed to make certain that the points of stimuli were properly located with respect to the ganglion. This procedure permits several determinations with little injury to the animal.

The tip of the foot of the preparation was connected to a light writing lever by means of thread. It was necessary to reduce the speed of the slide myograph by substituting a weight to draw the carriage for the spring that comes with the instrument. The key switch on the instrument made the stimulus and the rate of movement of the paper carriage was shown by recording a 100 d.v. tuning fork. The precautions indicated by Jenkins and Carlson (1903) and Maxwell (1907*b*) for avoiding experimental difficulties were followed in these experiments. The rates of nervous conduction were calculated from measurements of the times of the latent periods.

The average rate of nervous conduction of 64.2 cm. per second was obtained from 16 determinations made with 4 different preparations. The room temperature varied from 18–20° C. The animals were large, about 200 mm. long (*cf.* Richards, 1928), so that the average nerve length was about 25 mm. This length and the small probable error of ± 1.8 cm. per second indicate the reliability of the determinations.

However, if the rate of conduction through the ganglion be determined it is found to be 22 per cent. ± 2 per cent. slower than conduction along the nerve trunk distal to the ganglion. This strongly suggests the possibility that Fick[1] may have stimulated through a ganglion when in 1863 he found for the "nerve conduction" of *Anodon* the surprisingly low rate of 1 cm. per second. This rate is much slower than the rates for other molluscs are reported to be (*cf.* Jenkins and Carlson, 1903, and Rogers, 1927).

II.

The smaller size of the Eastern mussel, *M. edulis*, necessitated some modification of the technique. The shell was carefully opened by cutting the posterior retractor muscle and the pedal

[1] Quoted by Jenkins and Carlson (1903).

ganglion exposed by breaking through the tissue along the anterior retractor muscles of the foot. The small pedal ganglion is bright orange (Field, 1922), which aids in finding it. The animal was held in a small dish and then the tip of the foot was connected with a writing lever. By using the animal in the shell in this manner a less injured preparation is obtained. As the foot was too small to anchor near its tip, the rates had to be calculated from the latent periods of the reflex time from stimulating the foot distally and close to the body. As the contraction always occurs first at the base of the foot these times may be directly compared. A few determinations with the isolated foot gave essentially the same conduction rates, with a greater variability which was due to the technical difficulties involved with so small a preparation.

The average rate of conduction was found to be 92.9 cm. per second, from 12 determinations with 8 animals. Because the animals were smaller, the difference between the points of stimulation averaging about 18 mm., the probable error is greater, being 3.3 cm. per second. The room temperature averaged 24° C. and when this higher temperature in taken into consideration we see the rate in the two species of mussels is about the same.

The slowing of the conduction rate through the ganglion was determined by stimulating the nerves entering the ganglion, and the pedal nerve, stimulating in both cases close to the ganglion, and noting the differences in the latent periods of the responses. The distance between the points of stimulation averaged about 3 mm. The average time of conduction through the ganglion was 0.0087 sec. from 9 determinations with 7 different animals. This is about 2.7 times slower than the conduction along a nerve trunk would be, and further supports the suggestion of the possible cause of the slow rate found by Fick mentioned in the last section.

III.

If this delay in the rate of conduction through the ganglion is due to a synaptic resistance, it might be expected to be reduced when the ganglion is treated with strychnine. This proved to be the case, as five minutes after applying 1 : 500 strychnine SO_4

in sea water to the outside of the ganglion the difference dropped to an average of 0.003 second. This average was obtained with 6 tests with 4 animals. Before strychninization the average delay for the same animals was 0.008 second.

Strychnine does not "open the synapses" in the same way as with vertebrates, because unless the animal is stimulated the foot shows no contraction when its actions are recorded on a kymograph (Fig. 1). The threshold of excitation of skin, nerve

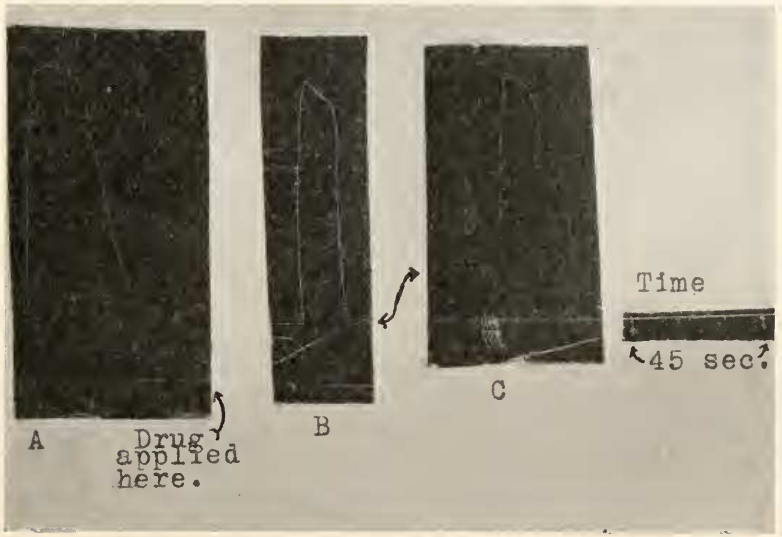

FIG. 1. The effect of applying strychnine to the pedal ganglion of *Mytilus*, as seen in the contraction of the foot. *A*, contraction before using the drug; *B*, contraction 6 minutes later; *C*, contraction 12 minutes later, which shows decreased irritability of the preparation.

and ganglion is greatly increased. Ten minutes after applying the drug the skin is barely sensitive to touch. If the foot is free to move and not attached to the recording apparatus, writhing movements are seen as soon as the drug is applied to the ganglion. These soon cease and the foot remains partly contracted. The circular muscles are contracted proportionally more than the longitudinal muscles. After an hour the foot becomes normally relaxed and quickly responds to stimuli.

Picrotoxin and philocarpine HCl (1 : 200) seem to have no

effect on the ganglion. Phenol 1 : 200 keeps the foot about half contracted, but its irritability remains the same as that of the control animal whose ganglion was washed with sea water. After the application of creatin 1 : 200 to the ganglion the circular muscles at the base of the foot remained contracted and prevented the foot from completely shortening when it was stimulated. The preparation seemed about as irritable as the controls. Thirty minutes after the application of caffeine 1 : 200 the foot became contracted to about one fourth of its normal size and exhibited rapid spontaneous movements. This may be due to some other and secondary effect rather than to the drug itself (*cf.* Maxwell, 1907*a*). Nicotine 1 : 200 may have reduced the sensitivity of the preparation slightly, but this was barely apparent.

Immediate squirming of the foot is seen when 1 : 200 atropine SO_4 is placed on the pedal ganglion. Two minutes later the foot is sensitive to stimuli but it can only contract half-way as the circular muscles at the base of the foot are tightly contracted and the tip of the foot remains flabby and relaxed. The foot becomes hypersensitive to stimuli, partially contracts and relaxes quickly, then a control foot seven minutes after the drug was applied. When it is relaxed the base is narrow from the contraction of the circular muscles, but the rest is greatly elongated as if the longitudinal muscles were forcibly relaxed. This seems to be a reversal of reciprocally acting muscles and somewhat resembles the "reversal" observed after atropine is injected into the body of caterpillars (Crozier, 1922). The effect of atropine on the mussel is similar to the effect of strychnine on the foot of *Chromodoris* (Crozier & Arey, 1919). It is very difficult to record the effect of atropine graphically as the increasing relaxation of the foot necessitates frequent readjustment of the base line and the slight pull on the foot of the writing lever initiates frequent movements of the foot. Such movements were rarely observed with the control preparations.

When a few drops of saturated solution of camphor in sea water were dropped on the pedal ganglion the foot immediately contracted and quickly relaxed. Within two minutes the threshold is so lowered that a jar of the table initiates a spasmodic con-

traction of the foot and adjacent musculature. Soon spasmodic movements of the foot are observed without any apparent stimuli. Six minutes later mantle spasms are observed. The foot becomes so irritable that it immediately contracts when it relaxes enough to barely touch the mantle or a gill. These twitchings are illustrated in Fig. 2. If the ganglion be isolated

FIG. 2. The effect of applying camphor to the pedal ganglion of *Mytilus*. (*Cf.* Fig. 3.)

by cutting the collaterals which connect it with the rest of the nervous system, the foot relaxes and remains relaxed unless stimulated (Fig. 3). Twenty minutes after camphor is applied, the foot is fully contracted to about one quarter of its usual size. Both the circular and longitudinal muscles are then tightly contracted. This effect of camphor resembles the effect of strychnine in lessening synaptic resistance in certain other animals.

The slower rate of conduction of the nervous impulse through the pedal ganglion of the mussel is abolished five minutes after the ganglion is bathed with strychnine, and the irritability of the foot neuromuscular mechanism is reduced. Atropine causes a reverse of the reciprocally acting muscles of the foot. Camphor seems to open the pathways so that an almost continuous discharge of nervous impulses keeps the foot in active movement until the foot is so contracted that it can hardly move. That this is not due to a stimulation of the ganglion cells is shown by

the relaxation and inactivity of the foot when the pedal ganglion is isolated from the rest of the nervous system.

The supra-œsophageal, pedal, and (in cephalopods) the stellate ganglia respond specifically to the application of strychnine or phenol (*cf.* Baglioni, 1905, Frölich, 1910, Moore, 1917). The injection of strychnine into *Limax* suppresses the phototropic

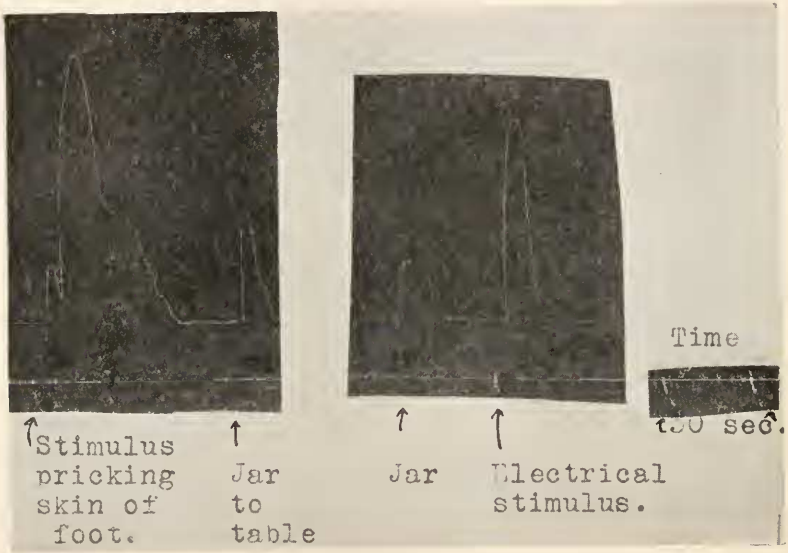

FIG. 3. The effect of camphor on the pedal ganglion of *Mytilus*, as seen in the contraction of the foot. The foot shows contraction only after direct stimulation of the preparation, when the cerebro-pedal connectives have been severed to isolate the pedal ganglion and nerve from the rest of the nervous system. (*Cf.* Fig. 2.)

circus movements of the animal without affecting the activity of the foot. Crozier and Federighi (1924) consider that this result is due to central "competition" between impulses, resulting in the release of pedal waves and in the maintenance of a turning posture.

Strychnine and atropine seem to affect different parts of the organization of the pedal ganglion of *Mytilus* and camphor seems to affect most of the neurones in the ganglion. Strychnine may do the same, but the effect may be masked by some direct or secondary anæsthetic action. These observations intimate caution in the interpretation of the possible locus of the strychnine

effect as synaptic. The chemical differences among the drugs used in this study imply chemical differences in the combination of the drugs with the nervous elements, but enough information is not yet available for a classification of the differences. The cephalopod is sensitive to caffeine, atropine and camphor (Moore, 1917). *Mytilus* is poisoned readily by atropine and camphor and less so by caffeine. The effect of strychnine is less pronounced on *Mytilus* than on *Chromodoris* (Crozier and Arey, 1919). The effect of drugs would place the mussel between the nudibranchs and the cephalopods, which is in agreement with taxonomy.

Summary.

The rate of nervous conduction in *Mytilus californianus* was found to be 64.2 ± 1.8 cm. per second at 18–22° C. and that of *M. edulis* was found to be 92.9 ± 3.3 cm. per second at 24° C. The rate of conduction through the pedal ganglion was much slower in both animals. Treatment of the ganglion by bathing it with strychnine abolished this delay. Applying camphor to the ganglion results in the foot exhibiting almost continuous movement with no apparent source of stimulation. These movements stop if the pedal ganglion is isolated by cutting the connectives just anterior to the ganglion. The longitudinal muscles of the foot are greatly relaxed and the circular muscles, especially at the base of the foot, contract when the foot is stimulated after applying atropine to the ganglion. This is just the reverse of the response of the foot of the control animal to stimulation. These observations are discussed with respect to the drug effects with other animals and to the use of strychnine as a test for the function of the synapse.

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